Spectroscopic resolution of the picosecond reduction kinetics of the secondary electron acceptor A_1 in photosystem I

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Abstract Forward electron transfer in photosystem I from *Synechocystis* sp. PCC 6803 has been studied in the picosecond time range with transient absorption spectroscopy in the blue and near-UV spectral regions. From the direct measurement, at 380–390 nm, of the reduction kinetics of the phylloquinone secondary acceptor A_1 and from the absence of spectral evolution between 100 ps and 2 ns, we conclude that electron transfer, from the chlorophyll *a* primary acceptor A_0, to A_1 occurs directly and completely with a time constant of about 30 ps.

Key words: Photosystem I; Electron transfer; Phylloquinone; Ultrafast spectroscopy; Ultraviolet

1. Introduction

Transmembrane charge separation in photosystem I (PS I), starting from the excited primary electron donor P700 (chlorophyll (Chl) *a* dimer), involves at least five electron acceptors: the primary acceptor A_0 (Chl *a*), the secondary acceptor A_1 (phylloquinone), and three (4Fe-4S) clusters called F_X, F_A and F_B (see [1,2] for reviews). The first two steps of charge separation have been studied by fs/ps spectroscopy monitoring absorption changes in the red (Q_Y) spectral region attributed to the reduction and reoxidation of A_0. According to these studies, A_0 was reduced with time constants of about 4–15 ps, accelerating with increasing excitation energy and with decreasing size of the antenna system [3–7]. At low levels of excitation and in the presence of the native antenna system of PS I, the kinetics of A_0 reduction are difficult to observe directly, but correspond presumably to the 30 ps overall decay time of the excitation in the antenna [4,8]. These effects indicate that transfer and partition of the excitation energy between the antenna pigments (mainly Chl *a*) and P700 affect the overall kinetics of primary charge separation. The time constant of reoxidation of A_0^- was reported to be about 30 ps [4,7,9], deviating from a previous estimate of about 200 ps [10]. These studies are complicated because several processes (spectral equilibration and primary and secondary electron transfer) take place on a similar time scale. In particular, energy transfer between spectrally different Chls (spectral equilibration) gave rise to strong absorption changes in the red spectral region. It was tried to correct for the energy transfer contributions by subtracting absorption changes measured in samples where charge separation was suppressed by preoxidizing P700; however, the underlying assumption that energy transfer is independent of the oxidation state of P700 is unlikely to be perfectly correct (see [2,11] for discussion), leaving some ambiguity with respect to the electron transfer kinetics cited above. In addition, it has not yet been experimentally proven whether the excess electron on A_0^- is transferred directly to A_1 or whether a further intermediate might be involved. This point is also relevant for determining the location of the phylloquinone A_1 which could not yet be resolved unambiguously by X-ray crystallography at a resolution of 4 Å [12]. Magnetic resonance data were consistent with 12 different positions of A_1 out of which two were judged to be sufficiently close to A_0 to allow for direct electron transfer in 30 ps [13].

Photovoltage measurements on a ps time scale could be fitted by a major electrogenic phase with a time constant $\tau = 22$ ps and a minor one with $\tau \approx 50$ ps [14]. The latter phase was suggested to reflect electron transfer from A_0^- to A_1, but unfortunately this method does not provide information on the chemical nature of the transient states.

The kinetics of A_1^- can be monitored directly in its near-UV absorption band around 380 nm [15,16]. In the study with the hitherto best time resolution (2 ns), the decay of A_1^- due to forward electron transfer to the iron-sulphur clusters was found to occur with time constants of about 10 ns (30% of the reoxidation of A_1^-) and 300 ns (70%), but the observed rise of the absorption due to the reduction of A_1 was still instrument limited [17]. Here we succeed in resolving the reduction kinetics of A_1 in the 380–390 nm range on a picosecond time scale. The result that the reduction of A_1 occurs with the same kinetics ($\tau \approx 30$ ps) as the reoxidation of A_0^- provides evidence of a direct electron transfer from A_0^- to A_1.

2. Materials and methods

2.1. Sample preparation

Monomeric PS I complexes were extracted from *Synechocystis* sp. PCC 6803 according to [18] and diluted with a buffer containing 20 mM Tricine (pH 8), 0.03% *n*-dodecyl-β-D-maltoside, 10 mM sodium ascorbate and 400 μM 2,6-dichlorophenolindophenol. The optical density of the sample in the red absorption maximum was 1.13 (optical path length 1 mm) and 1.28 (optical path length 2 mm) for measurements on the ps and ns time scale, respectively.

2.2. Femtosecond absorption spectroscopy

Multicolour transient absorption spectroscopy on the picosecond time scale was performed with a pump-probe spectrometer operating at 30 Hz described previously [19,20]. For the present experiments, the instrument was set up as follows. The pump pulses (fwhm ∼30 fs) were centred at 563 nm and focused to ∼50 μm in the sample. The energy of the pump pulses was adjusted to 8–10 nJ which corresponds

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Abbreviations: ΔA, absorption change; Chl, chlorophyll; PS I, photosystem I

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to ~2 excitations per reaction centre. The chirp in the continuum probe pulse was minimised at ~400 nm. The continuum generation conditions were optimised for high signal to noise in the wavelength region below 400 nm, which allowed us to exploit the spectra down to 380 nm. Transient spectra were recorded at intervals of 7 ps on a 200 ps full time scale. The spectra were measured with a CCD camera placed in the focal plane of a polychromator, where the pixels corresponding to ~1 nm were binned. Absorption difference spectra were obtained by subtracting the absorption spectrum recorded prior to the pump pulse from transient absorption spectrum recorded after the pump pulse. The sample was continuously moved in a direction perpendicular to the beams. Measurements were performed at room temperature and a fresh sample was taken after ~45 min of signal averaging. The ground state spectrum was recorded before and after each experiment and was found to be unchanged.

Analysis of the data in terms of decay associated spectra was performed with a procedure described previously [21], involving singular value decomposition and deconvolution with the instrument response function.

Nanosecond absorption spectroscopy was performed with a set-up described in [22] using 300 ps pulses of about 1 mJ/cm^2 at 532 nm for excitation (repetition rate, 1 Hz) and the relatively flat top of a 50 μs Xe flash as measuring light. The instrumental time resolution was about 2 ns. For further details, see [17].

3. Results and discussion

Fig. 1 shows absorption difference spectra in the Soret region at various delay times. The earliest recorded spectrum (3 ps) is characterised by a bleaching with a maximum at 439 nm and an induced absorption at $\lambda > 451$ nm. The shape of the bleaching is very similar to that of the ground state absorption of the sample (not shown), consistent with excited Chl *a* being predominantly present at this time. The main features of the spectral evolution on the picosecond time scale include a reduction and blue shift of the main bleaching, a fast decay of the induced absorption around 460 nm, and in particular the appearance of a an induced absorption at $\lambda < 408$ nm.

Global analysis demonstrates that the spectral evolution can be well described by two exponentials and a constant:

$$\Delta A(\lambda, t) = \Delta A_1(\lambda)\exp(-t/\tau_1) + \Delta A_2(\lambda)\exp(-t/\tau_2) + \Delta A_\infty(\lambda) \quad (1)$$

Here $\Delta A_1(\lambda)$, $\Delta A_2(\lambda)$ and $\Delta A_\infty(\lambda)$ are the decay-associated spectra. A best fit was found with time constants of 7 and 28 ps.

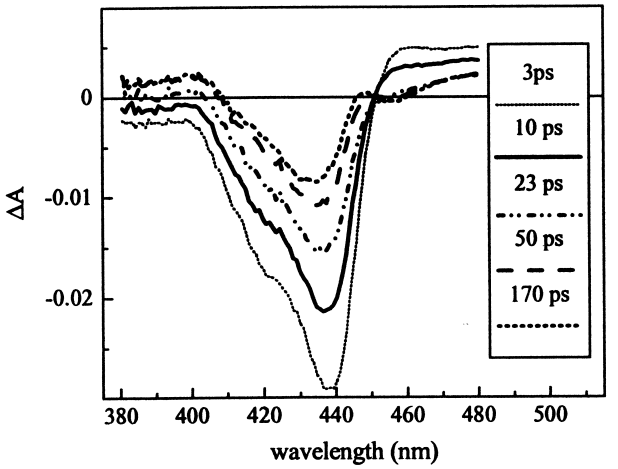

Fig. 1. Absorption difference spectra at various delay times.

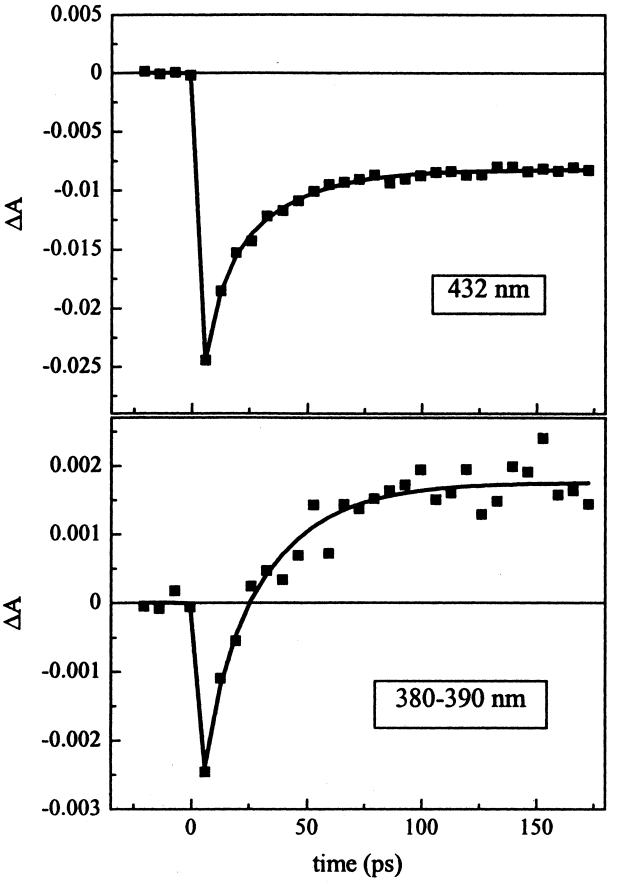

Fig. 2. Kinetics at 432 nm and average over 380–390 nm. The solid lines are fits according to Eq. 1 with the following parameters: τ_1 = 7 ps, τ_2 = 28 ps, ΔA_1(432 nm) = −0.0147, ΔA_2(432 nm) = −0.0131, ΔA_∞(432 nm) = −0.0081, ΔA_1(380–390 nm) = −0.0018, ΔA_2(380–390 nm) = −0.0044, ΔA_∞(380–390 nm) = 0.0018.

The 7 ps component presumably reflects spectral equilibration, which is known to take place on this time scale [8,23,24], excitation annihilation, which is expected under our experimental conditions, and trapping ($P700^+A_0^-$ formation). The fit results for the time constant and the spectrum of the 7 ps component depended somewhat on the fit conditions, presumably because of the large spacing between the time points. No effort was made to better resolve this phase.

Fig. 2 shows the measured transients and the fit functions at 432 nm (the maximum of the asymptotic bleaching) and for the 380–390 nm region, where reduction of the phylloquinone A_1 should show up as an absorption increase ($\Delta\varepsilon \approx 10$ mM^{-1} cm^{-1} [15–17]). Absorption changes due to the formation of $P700^+$ are comparatively weak ($\Delta\varepsilon \approx 3$ mM^{-1} cm^{-1}, averaged between 380 and 390 nm [25]), and the reduction of A_0 is expected to be accompanied by a bleaching in that region ($\Delta\varepsilon \approx -13$ mM^{-1} cm^{-1} for the reduction of Chl *a* in dimethylformamide [26]). Therefore we attribute the rise of the 380–390 nm transient above zero with $\tau \approx 30$ ps[3] to the reduction of A_1.

[3] As the time constant of 28 ps was obtained by a global analysis of the transients at all wavelengths, it might have been biased by the large signals in the 400–450 nm region. A separate fit to the 380–390 nm transient in Fig. 2 yielded τ_1 = 5 ps, τ_2 = 30 ps, $\Delta A_1 = -0.0018$, $\Delta A_2 = -0.0043$, $\Delta A_\infty = 0.0019$, in good agreement with the result of the global analysis (see legend of Fig. 2).

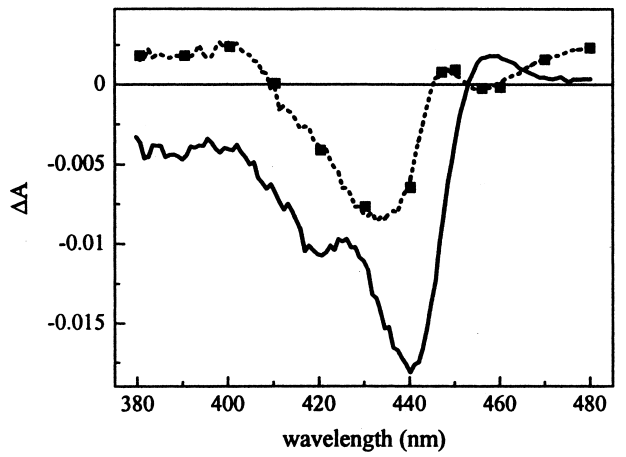

Fig. 3. Decay associated spectra of the 28 ps component (solid line) and the asymptotic component (dashed line). Solid squares: spectrum of the 'initial' absorption changes from nanosecond experiments, obtained by back-extrapolation to t=0 of transients measured on a 400 ns time scale with a time resolution about 2 ns (see [17] for details); the entire spectrum was multiplied by a factor 0.86 for the ease of comparison with the asymptotic component on the ps scale.

The spectrum associated with the 28-ps phase (Fig. 3, solid line) is dominated by a negative double band (around 420 and 440 nm) which resembles the A_0^- minus A_0 difference spectrum deduced from measurements on a ns time scale [27]. Hence our data are consistent with the conclusion from studies in the red spectral region that A_0^- is reoxidised with $\tau \approx 30$ ps [4,9]. As some trapping is likely to take place on the 30 ps time scale under our excitations conditions (part of the PS I complexes absorbs only one photon) [8], decay of excited states may contribute to the spectrum of the 28 ps component (Fig. 3, solid line)[4]. The negative plateau at 380–400 nm, reflecting an absorption increase with $\tau = 28$ ps, contains presumably a contribution due to the reoxidation of A_0^- (and possibly a contribution due to the decay of excited states), in addition to that due to the reduction of A_1 (see above).

The spectrum of the asymptotic phase (dotted line in Fig. 3) is similar to spectra attributed to the state $P700^+A_1^-$ in studies on ns and μs time scales [16,28,29]. Moreover, the spectrum is in excellent agreement with the initial spectrum obtained with a nanosecond spectrometer (time resolution 2 ns) on the same sample material (Fig. 3, solid squares; note that the absolute amplitudes of the two spectra were somewhat different due to different concentrations and excitation conditions). This demonstrates that no significant spectral evolution occurred in the range from 100 ps to 2 ns. We conclude that A_1 is completely reduced by direct electron transfer from A_0^- with a time constant of about 30 ps. This result supports a position of A_1 close to A_0, as suggested in [13].

[4] During the review process, we learned that R.E. Blankenship and coworkers (personal communication) observed a phase with similar lifetime and difference spectrum under low intensity illumination conditions and attributed it to the trapping processes.

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